https://doi.org/10.47262/BL/9.1.20230220

Research article
2023 | Volume 9 | Issue | Pages 40-47

ARTICLEINFO

Received
February 23, 2023
Revised
April 06, 2023
Accepted
April 12, 2023

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Keywords
Computational drug design
Bioinformatics
In silico structure prediction
Schizophrenia
SP4

How to Cite
Hameed I, Zubair A, Nazir A, Shahid K, Aimen S, Khan MI, Sabir S, Khan MF, Raza A. Computer Aided Drug Design against schizophrenia by targeting SP4. Biomedical Letters 2023; 9(1):40-47.

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Computer-aided drug design against schizophrenia by targeting SP4

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Abstract

Schizophrenia (SZ) is a mental disorder and affects ~1% of the worldwide population. It is considered a chronic and severe condition that impacts the thoughts, emotions, and behavior, of the patient often leading to a distortion of reality. Numerous computational techniques such as threading technique, homology modeling technique, and *ab initio* technique were applied for 3D structure prediction of the selected SZ protein SP4. The 3D predicted structures of SP4 were further evaluated and validated by utilizing Anolea, ProCheck, and Errat evaluation tools. Interestingly, it was observed that the overall quality factor of the selected structure was 77.542%. The predicted structure of SP4 showed 3.97% residues in the outlier region of Ramachandran plot while 96.03% in the allowed and the favored region of the evaluated plot. The study of molecular docking analyses was done to identify the compounds against SZ by targeting SP4. Moreover, the scrutinized compounds showed the least binding energy of -10.1 Kcal/mol. The highest binding affinity was observed among the binding residues (Leu-199, Ala-275, Gly-262, Leu-198, Thr-333, Ser-334, Leu-339, Ala-206, Leu-208, Gly-281, Ile-207, Val-283, Pro-286, and Ala-287). The scrutinized molecules from the selected library may have the ability to regulate the activity of SZ by targeting SP4. The scrutinized molecules can behave as a potential compound and

the 3D predicted structure of SP4 is reliable for structural insights and functional analyses.

Introdu

Schizophrenia (SZ) is a mental disorder and affects ~1% of the worldwide population. It is a chronic and severe condition that impacts a person's thoughts, emotions, and behavior, often leading to a distortion of reality. It typically begins in early adulthood and usually has a severe impact on the ability of a person [1]. It is a complex disorder with a multifactorial etiology that involves genetic, environmental, and neurobiological factors. SZ is hard to diagnose and also occurs worldwide. SZ is considered the foremost neurological disorder and also consider the leading cause of suicide among patients in underdeveloped and developed countries [2].

The symptoms of schizophrenia are divided into cognitive, negative and positive categories. The cognitive symptoms of SZ include problems with decision-making, attention and memory [3]. The negative symptoms of SZ patients refer to the experiences that took away the ability of a person to function including lack of motivation and emotion, and difficulty with social interactions. The positive symptoms of SZ patients refer to experiences that are added to the perception of a person towards reality such as hallucinations and delusions [4]. The causes of SZ are still unknown however the researchers suggest environmental, a combination of genetic and brain chemistry may involve. There are numerous risk factors involved in SZ and to develop SZ includes exposure to viruses, family history of the disorder, malnutrition during pregnancy and drug use during adolescence [5].

crucial protein involved in the control of gene expression. SP4 plays a key role in regulating gene expression in the brain. It is particularly significant to develop the prefrontal cortex, an area of the brain responsible for decision-making, problem-solving, and social behavior. SP4 belongs to SP1 protein family of transcription factors; it possesses a DNA-binding domain that binds to promoter elements rich in GC. Within the brain, SP4 is highly concentrated and serves significant roles in the formation and operation of neuronal circuits, while also contributing to the pathogenesis of neurological and psychiatric conditions [6].

It has been previously discovered that SP4 single nucleotide polymorphisms (SNPs) are linked to SZ, bipolar disorder and MDD in the White population. Additionally, rare deletion and copy number variations of *SP4* have also been linked with SZ. SP4 plays a crucial role in the cerebellar granule neurons development by facilitating the activity-dependent pruning of dendritic processes [7].

SP4 are more likely to develop schizophrenia than those without these variations. These genetic mutations can disrupt the normal functioning of SP4, leading to problems in the development and functioning of the prefrontal cortex. Environmental factors such as stress, trauma, and drug use can also affect the regulation of SP4 expression. Exposure to stress can increase the expression of SP4 in the brain, which can contribute to the development of SZ. Similarly, certain drugs, such as cannabis, can also disrupt SP4 expression and increase the risk of developing this disorder. SP4 plays a critical role in the development of SZ.

Genetic mutations and environmental factors can disrupt the normal functioning of SP4, leading to problems in the development and functioning of the prefrontal cortex. Understanding this link between SP4 and schizophrenia is critical for the development of effective treatments and interventions for this complex mental illness [8]. There has been impressive progress reported in computational drug design and immuno-informatics [9-22] over the last two decades. Various problems in life sciences and biological sciences have been solved by using the different applications and approaches of computational biology [2]. The current study utilized the computational approach of molecular docking studies to reveal the novel scrutinized molecules against SZ by targeting SP4. The 3D experimentally solved structure of the target protein (SP4) by applying a Nuclear Magnetic Resonance (NMR) structural approach and X-ray crystallographic structural approach was not yet predicted. The 3D model of the target protein SP4 was predicted by applying various approaches of bioinformatics and the generated structure showed reliable results.

Materials and Methods

The amino acid sequence of the target protein SP4 in FASTA format with the accession number O91532 was retrieved by using Uniprot Knowledgebase database. In current struggle, the 3D structure prediction of SP4 was done by using homology modeling, threading and *ab initio* approaches on a workstation of HP core-I-9. The structure prediction experiment was followed by using the approach of molecular docking analyses for virtual screening. The sequence of the selected protein SP4 was analyzed and subjected for suitable template hits to BLASTp by using thee Protein Data Bank (PDB) [23] database of 3D structures. An automated and command-based program MODELLER 9.24 [24], was employed to predict the 3D structure of the selected protein. The suitable templates were analyzed and selected based on query coverage and the selected templates were then utilized for the 3D structure prediction of SP4 [25]. Numerous tools (Anolea [26], ProCheck [26], and ERRAT [27]) for the evolution of the predicted structures were utilized to verify the reliability of the predicted structures.

The commercial molecular library of ZINC compounds was used to scrutinize, evaluate and analyze the conserved binding domain of the selected protein. The optimization of the bond length and bond angles of the scrutinized compounds and the 3D predicted structure of SP4 was done by utilizing the ChemDraw Ultra and UCSC Chimera 1.9 respectively. The ligands available in the library were minimized to optimize the geometry of all the ligands and molecular docking analyses were done for all the ligands of the selected library by using AutoDock Vina and AutoDock tools docking software [28]. The drug properties of all the selected scrutinized molecules were calculated such as H-bond donors, H-bond acceptors, and a number of rotatable bonds through applying the PubChem [29]. The reliability of the scrutinized compounds was measured through Lipinski's rule of five [30] and the values were calculated by employing the mCule servers. The carcinogenicity, toxicity and mutagenesis were also measured for the selected molecules. The docked complexes of the ligand and the selected target protein were analyzed for binding regions and visualized through UCSC Chimera 1.9 and Ligplot. The absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties also play a key role in the reliability of the predicted structures and the ADMET properties were also calculated for all the selected top-ranked compounds by using the admetSAR online server.

Results and Discussion

The objective of current *in silico* analyses of SZ by targeting SP4 was to identify the conserved binding region of the selected protein along with the novel compounds through molecular docking analyses. The suitable templates retrieved through BLASTp for the 3D structure prediction of SP4 having similarity, identity, score, query coverage and E-value were selected (**Table 1**). Suitable templates solved through X-ray crystallography and NMR was utilized for the structure prediction of SP4. It was observed that the selected scrutinized templates did not show reliable results for the 3D structure prediction through the homology modeling approach. The generated structures through the homology modeling approach were evaluated and were not considered for further analyses. Threading and *ab initio* approaches were utilized to predict the reliable structure of the selected target protein. The predicted 3D structures through threading and *ab initio* approaches showed reliable results.

The comparative analyses of the generated results of the predicted structures of SP4 were plotted for the selection of suitable structures and the most potent structure for SP4 was selected from the generated graph (**Fig. 1**).

Numerous structures were generated and evaluated through selected evaluation tools, and the utilized tools revealed the accuracy and the reliability of the predicted structures (**Fig. 2**). The outlier region along with the allowed region and favored region of 3Dstructures of SP4 were analyzed through Ramachandran plot. It was observed that 77.5% of the residues were present in the allowed region and the favored region of the generated plot. However, the outliers were also calculated and 2.97% of the residues were observed in the outlier region. The reliability of the predicted structure also depends on the overall quality factor of the structure and 96.03% of the overall quality factor of the protein structure was observed. The evaluation results built the confidence to use the predicted structure for further analyses of computer-aided drug design.

Furthermore, the study of molecular docking analyses for the selected compound showed differences and variations in the observed binding energy of all the compounds from the library. The analyses of the molecular docking were performed with thirty different poses followed by 80 runs for each compound and the complexes were saved. Interestingly, it was observed that the suitable poses of the analyzed complexes showed the highest binding affinity and least binding. The scrutinized molecule showed efficient binding against the selected protein of SZ (SP4) (**Table 2**).

The study of molecular docking analyses was critically visualized and analyzed based on the effective binding energy, drug properties of the selected compounds, and efficient binding affinity (Table 3). The scrutinized compound showed cyclic rings (**Fig. 3**) with significant properties. The

Description	Accession ID	Total Score	Query Coverage	E-Value	Max-Identity
Chain A Kruppel-like factor 5	2EBT	184	12%	9e-34	126
Chain A Kruppel-like factor 4	6VTX	121	10%	2e-32	121
Structure of Klf4 zinc finger DNA binding domain in complex with methylated DNA	4M9E	121	11%	2e-32	121
Crystal structure of zinc finger domain of Klf4 bound to its target DNA	2WBS	121	11%	2e-32	121

Table 1: The suitable templates for comparative modeling for SZ by targeting SP4 with their query coverage, accession ID, E-value, total score, and identity

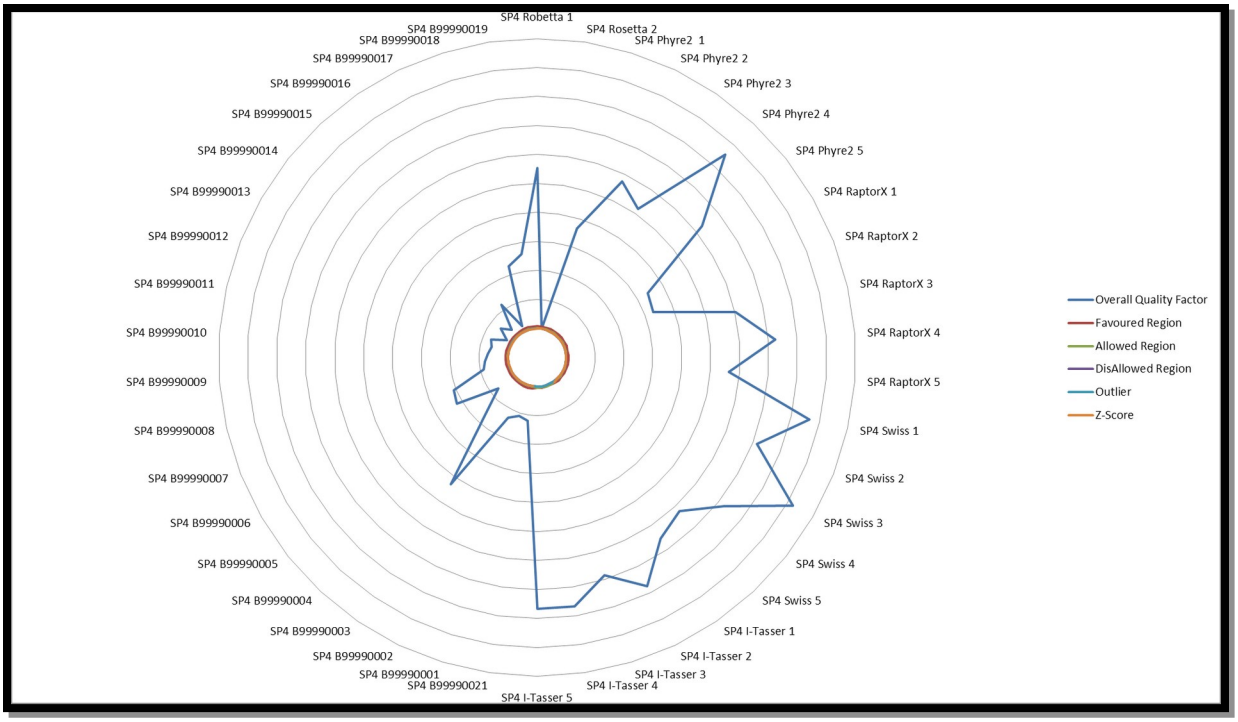

Fig. 1: The comparative assessment plot of the predicted structures of SP4 showed overall quality factor, favored region, allowed region, and outlier regions.

Table 2: Ligand efficacy and binding energy of the selected compound through molecular docking analyses

Properties	SP4
Estimated inhibition constant, Ki (μM)	36.66
Ligand efficiency	-0.51
Unbound system's energy (kcal/mol)	-0.49
Final intermolecular energy (kcal/mol)	-10.10
Torsional free energy (kcal/mol)	3.99
Estimated free energy of binding (kcal/mol)	-6.9
Binding residues	Leu-199, Ala-275, Gly-262, Leu-198, Thr-333, Ser-334, Leu-339, Ala-206, Leu-208, Gly-281, Ile-207, Val-283, Pro-286 and Ala-287

a scrutinized compound may be able to work as an anti-schizophrenic by targeting SP4.

The tools for molecular docking analyses were used and the scrutinized top-ranked complexes of SP4 showed the least binding energies. Minimal fluctuation and variations were observed regarding binding energy and the stability of the scrutinized compounds based on the conserved binding affinities was observed for the docked complexes.

It was also analyzed and observed that the scrutinized complexes against SP4 showed satisfactory results by applying the default parameters. Interestingly, it was observed that the scrutinized compound bound at the conserved site of SP4. Computational analyses and studies suggested that the scrutinized compounds bound at binding residues may lead to revealing the least binding energy (**Fig. 4**).

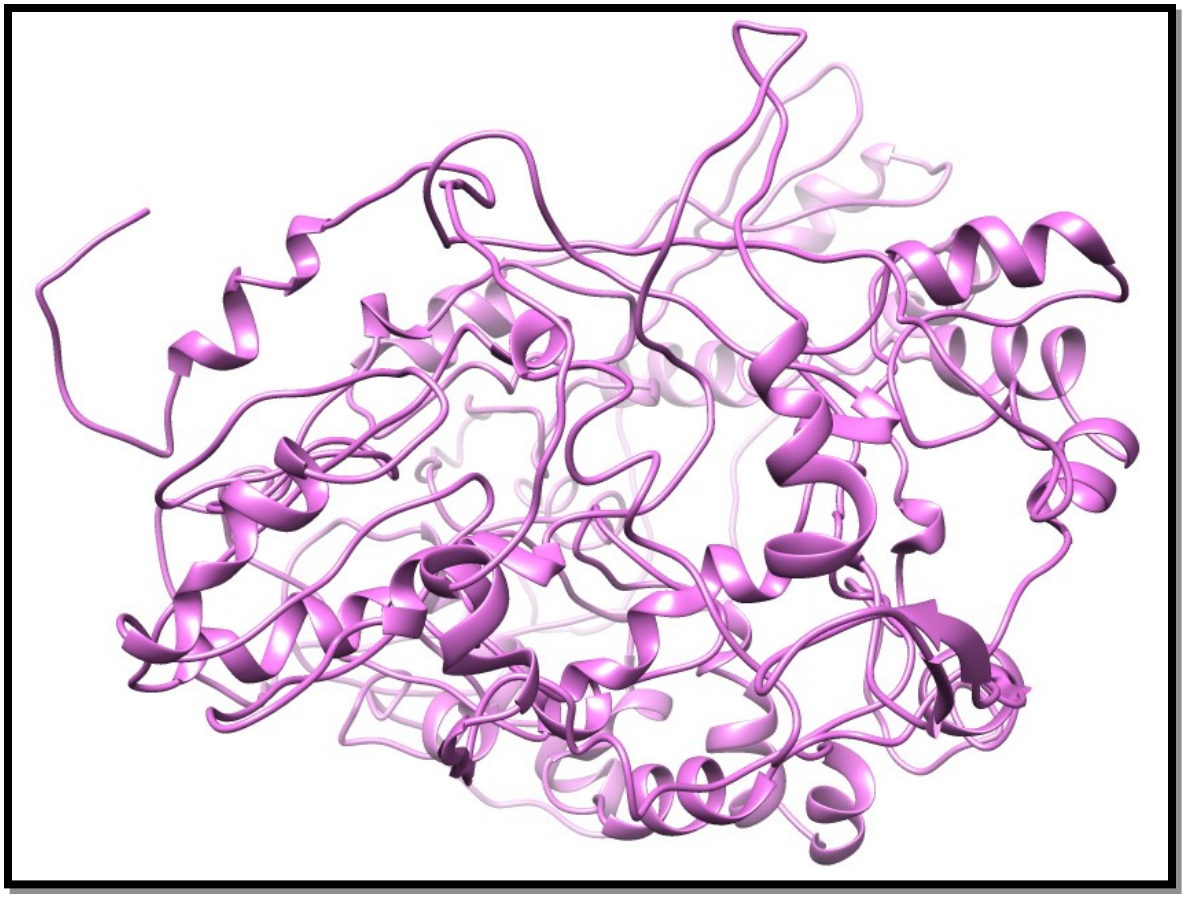

Fig. 2: The predicted 3D structure of SP4

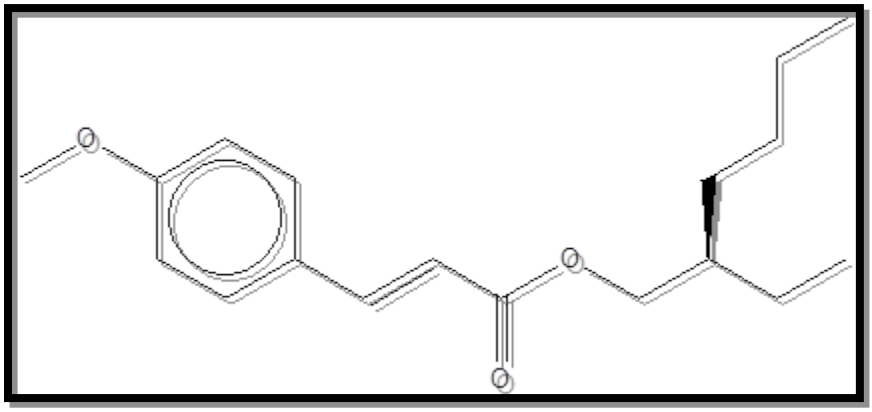

Fig. 3: The scrutinized compound showed cyclic rings. 2D structural view of the scrutinized compound (ZINC1633887).

The process of drug design is a costly and time-consuming method [17]. Hence, different approaches and numerous techniques of *in silico* analyses have been performed for the development of a drug [19]. The advancement in computational techniques has the importance to decrease the time and also to minimize the side effects [31].

The scrutinized compounds have been analyzed and the 2D structures of the scrutinized compounds were further evaluated to check the efficacy and oral bioavailability [32]. The scrutinized

compounds showed a reliable degree of ADMET properties. The included mathematical models were fish toxicity, cytochrome P450 2D6 inhibition, carcinogens, Ames

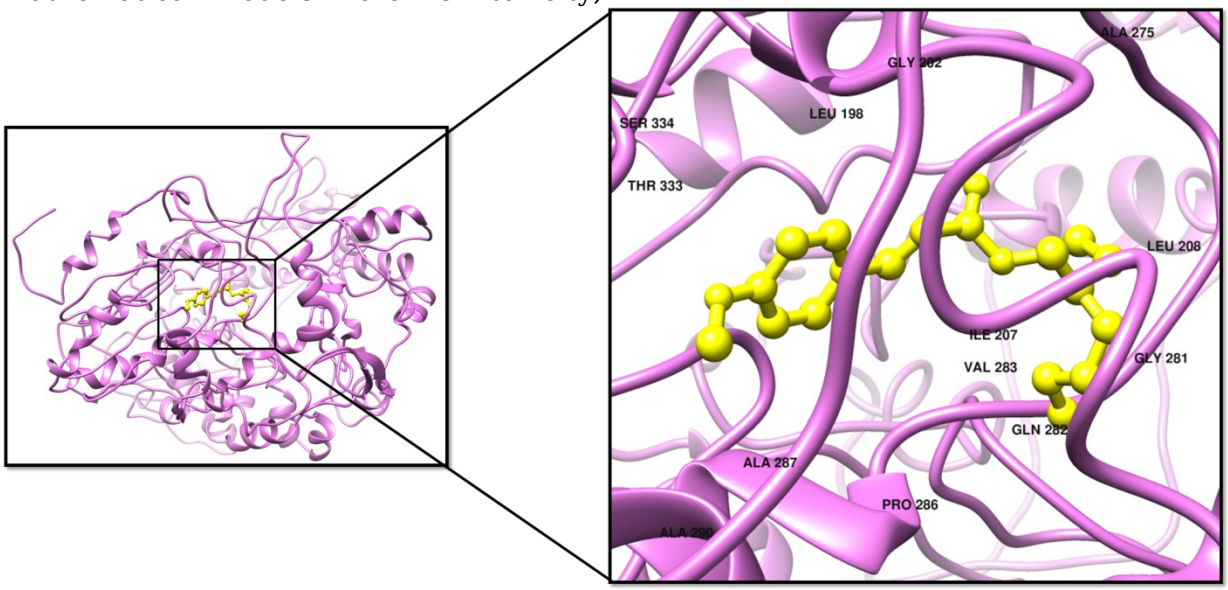

Fig. 4: The observed residues of interactions against scrutinized compound (ZINC1633887) with SP4.

toxicity, blood-brain barrier penetration, aqueous solubility [LogS], honeybee toxicity, human intestinal absorption, acute oral toxicity, and Caco2 permeability were analyzed for the selected compounds. Various toxicity analyses were performed (Table 3). The ADMET properties analyses of the selected compounds were cross-verified and found similar results. The toxicity analyses and the observed toxicity calculations help in the evaluation of different pollutants, metabolites and intermediates [15].

Table 3: The biological and drug-able properties of the scrutinized compound

Ligand properties	ZINC000001633887
Molecular Formula	C18H26O3
Hydrogen bond donor	00
LogP	4.468
Rings	1
Hydrogen bond acceptor	3
Heavy Atoms	21
Molecular weight (g/mol)	290.403
tPSA	35
Rotatable bonds	09
Hetro Atoms	3
Blood-brain barrier (BBB) (probability)	0.8900
Human intestinal absorption (HIA) (probability)	0.7000
Caco2 permeability (probability)	0.8668
CYP450 2D6 inhibitor (probability)	0.9400
Carcinogens (probability)	0.9500

The solubility of water molecules at 25°C was also calculated for the scrutinized compounds and it was observed that all the scrutinized compounds showed water solubility. The scrutinized molecules showed the least value of LogP. Extensive computational analyses and literature surveys suggested that the scrutinized.

Compounds must satisfy the selected parameters of highest binding affinity and least binding energy. By employing the utilized parameters, it was observed that the scrutinized molecules have the potential to use against SZ to target SP4. The results observed after molecular docking analyses revealed that Leu-199, Ala-275, Gly-262, Leu-198, Thr-333, Ser-334, Leu-339, Ala-206, Leu-208, Gly-281, Ile-207, Val-283, Pro-286 and Ala-287 were potent binding residues.

Conclusion

In conclusion, the current effort suggested that the reported molecule showed efficacy in hallucination and SZ treatment by targeting SP4. The

scrutinized compound (ZINC1633887) showed efficiency against SZ by targeting SP4 by applying extensive *in silico* approaches. Computational structure prediction, virtual screening, and molecular docking analyses of SP4 revealed the least binding energy, drug properties and high binding affinity based on the utilized parameters. The potent residues (Leu-199, Ala-275, Gly-262, Leu-198, Thr-333, Ser-334, Leu-339, Ala-206, Leu-208, Gly-281, Ile-207, Val-283, Pro-286 and Ala-287) of interaction between SP4 and (ZINC1633887) were observed and may be significant for site-directed mutagenesis. Though numerous divergences exist among the baseline population, trial-methodology studies and bioinformatics-based analyses seem to be sufficient to conclude that the reported compound may be the better option for SZ treatment.

Acknowledgments

The authors are thankful to Ms. Shahista Shaoor and Mr. Muhammad Farhan Shafique from Muhammadan Laboratory of Computational Biology, Pakistan for their support and guidance throughout this project.

Conflict of interest

The authors declare no conflict of interest.

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